Nanostructures

Self-assembled domain patterns

The ordered domain patterns that form spontaneously in a wide variety of chemical and physical systems[1,2] as a result of competing interatomic interactions can be used as templates for fabricating nanostructures. Here we describe a new self-assembling domain pattern on a solid surface that involves two surface structures of lead on copper. The evolution of the system agrees with theoretical predictions[3–5], enabling us to probe the interatomic force parameters that are crucial to the process.

The two structures showing this remarkable behaviour are a disordered Pb/Cu surface alloy, produced by deposition of lead vapour onto clean Cu(111), and a lead-overlayer phase, which is produced by continued deposition after the alloy structure has covered the surface[6–8]. Figure 1a–g shows a sequence of low-energy electron microscope (LEEM) images in which the overlayer structure (bright) grows over the surface alloy structure (dark) during lead deposition at a temperature of 673 K. The most striking feature of this sequence is the evolution of a pattern from circular islands (average diameter, 67 nm) to stripes and then to circular holes within the lead-overlayer matrix.

This sequence of domain patterns, referred to as droplets, stripes and inverted droplets, respectively, has been predicted to be a general property of domain formation on solid surfaces[3–5,9,10]. To our knowledge, these results represent the first unambiguous observation of the expected sequence of domain patterns with changing area fraction during deposition, and agree with the predicted positions[5] of the droplet-to-stripe phase transitions for short-range attractive and long-range dipolar interatomic interactions on solid surfaces.

One reason why these domain patterns form in this system is the high mobility of the islands (Fig. 1a), which contain many thousands of atoms and can move by as much as a micrometre before being incorporated into the more tightly packed droplet structure (Fig. 1b). This mobility allows patterns to form that have hundred-nanometre periodicity and allows the underlying interatomic interactions to be determined.

As the islands move in the force fields of the other islands, the island trajectories contain information about the force fields. Droplet trajectories are consistent with the expected long-range dipolar repulsion. Because magnetic interactions are not relevant in this system and we can rule out electrostatic interactions from measurements of work-function differences, we propose that elastic interactions arising from a surface-stress difference between the alloy and overlayer structures are responsible for the stabilization of domain patterns[9,10].

The behaviour of the patterns at fixed coverage and varying temperature is also qualitatively consistent with theory[3]. For both droplets and stripes, the feature size decreases with increasing temperature. The most likely reason for this is a reduction in the domain-boundary free energy with increasing temperature. For droplets and inverted droplets, reducing the temperature also allows improvement in the long-range order. Figure 1h is a LEEM image showing the degree of long-range order that can be obtained in the inverted-droplet phase.

If self-assembled structures are to be useful as nanostructure templates, two-dimensional, self-assembled patterns need to be stable at room temperature and resistant to air exposure. This is the case for our Pb/Cu system. Features that correspond to lead-overlayer droplets can be clearly seen in atomic-force-microscopy images taken after cooling to room temperature and 2 hours exposure to air (Fig. 1i).

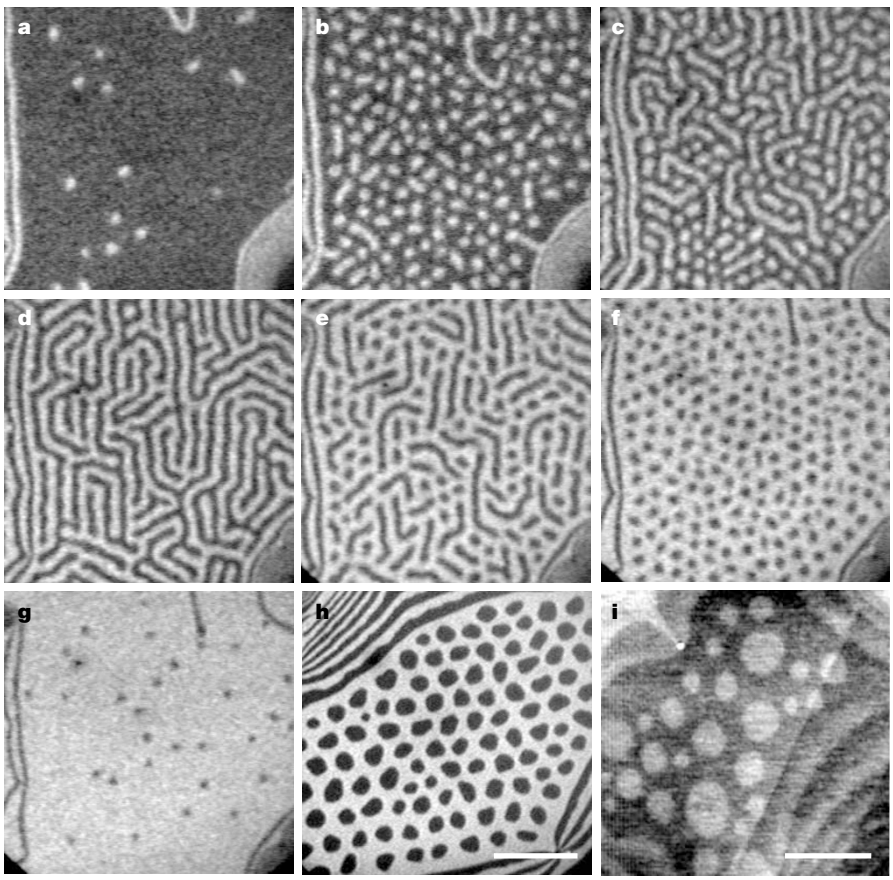

Figure 1 Self-assembly of Pb on Cu(111). Low-energy electron micrographs of the Cu(111) surface at 673 K with different area fractions of the lead-overlayer phase (bright) in the surface alloy phase (dark). **a–g,** Area fractions 0.03, 0.28, 0.35, 0.50, 0.65, 0.73 and 0.95, respectively. The domain pattern evolves from circular islands (droplets) to stripes, to vacancy islands (inverted droplets) with increasing lead coverage. **h,** Ordered droplet configuration at 623 K. Scale bar, 0.5 μm. **i,** Atomic-force micrograph of a droplet pattern after cooling down to room temperature and 2 hours of exposure to air. Scale bar, 0.3 μm.

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Malaria

Cooperative silencing elements in *var* genes

Each *Plasmodium falciparum* malaria parasite carries about 50 *var* genes from a diverse family that encode variable adhesion proteins on the infected red blood cells of the host[1–3], but individual parasites single out just one *var* gene for expression and silence all the others[4,5]. Here we show that this silencing is established during the DNA-synthesis phase (S phase) of the cell cycle and that it depends on the

cooperative interaction between two elements in separate control regions of each *var* gene (the 5′-flanking region and the intron). This finding should help to clarify the mechanisms by which parasites coordinate the silencing and activation of *var* genes that are responsible for antigenic variation in malaria.

The proteins (PfEMP1) encoded by the *var* genes enable red blood cells infected with *P. falciparum* to adhere within the circulatory system, thereby avoiding destruction by the spleen. Different PfEMP1 forms can attach to different host molecules, leading to patterns of sequestration that produce disease complications such as cerebral malaria or the severe malaria of pregnancy[6,7]. Because the exposed PfEMP1 molecules are subject to antibody attack during infection, *P. falciparum* parasites produce subpopulations with antigenically diverse PfEMP1 forms which must be continually chased by the immune response. The underlying switches in *var*-gene expression that result in this process of antigenic variation have yet to be understood.

Silent *var* promoters become transcriptionally active when removed from their chromosomal context[8], suggesting that further control elements in the intact *var* gene are required to control or silence expression. Possible locations for such control elements are in the conserved *var* introns, which separate the two exons of all *var* genes and have sequences that are found in many heterogeneous sterile transcripts in erythrocyte-stage parasites[2].

To test whether *var* introns function in gene control, we inserted a representative intron sequence (*int*) into two different plasmids containing a luciferase (*luc*) reporter driven either by a *var* promoter sequence or by a promoter sequence from the unrelated gene *hrp3*, which encodes histidine-rich protein 3 (pVLH and pHLH, respectively; Fig. 1a). Transfection experiments with pVLH and pVLH/*int* indicated that the presence of *int* downstream of *luc* strongly repressed the luciferase signal (Fig. 1b, left), irrespective of *int* orientation. In contrast, the presence of *int* in the pHLH/*int* plasmid caused no detectable change in the reporter activity (Fig. 1b, right). The fact that the *var*, but not the *hrp3*, promoter was affected indicates that *int* repression is specific and occurs by cooperative action with the upstream *var* 5′-flanking region.

These initial experiments revealed strong repression, but not complete silencing, of *luc* expression from pVLH/*int* (Fig. 1b). Transition through S phase of the cell cycle is known to be required for the assembly of silent chromatin structures in other organisms[9]. Because parasites continually take up DNA during their growth in plasmid-loaded erythrocytes[10], our experiments may have included heterogeneous populations of plasmids acquired before and after S phase, leading to incomplete silencing of the *var* promoter.

To test this possibility, we measured *luc* expression from homogeneous plasmid populations before and after transition through S phase. Assays done 18 h after invasion of plasmid-loaded erythrocytes (before the onset of S phase) confirmed that *luc* was expressed without repression (Fig. 1c, left). After their maturation to mature schizont stages, we separated transfected parasites from plasmid-loaded erythrocytes using Percoll/sorbitol gradients. Returning these parasites to fresh cultures with plasmid-free erythrocytes eliminated further plasmid uptake after re-invasion and ensured that all plasmid DNA in the parasites had been through S phase. Complete reporter silencing from pVLH/*int* was then evident (Fig. 1c, right). We detected no silencing in control transfections with the original pVLH, or with a pVLH/*hsp* construct in which the *int* sequence was replaced with a *P. falciparum hsp86* intron of similar size (Fig. 1c, right).

The regulatory elements and S-phase-dependent silencing we identify here implicate cooperative DNA-binding complexes and modifications of chromatin structure in the control of *var*-gene transcription. Experimentally manipulable plasmid systems that incorporate mechanisms for *var* control may offer a useful approach by which to identify and characterize these complexes.

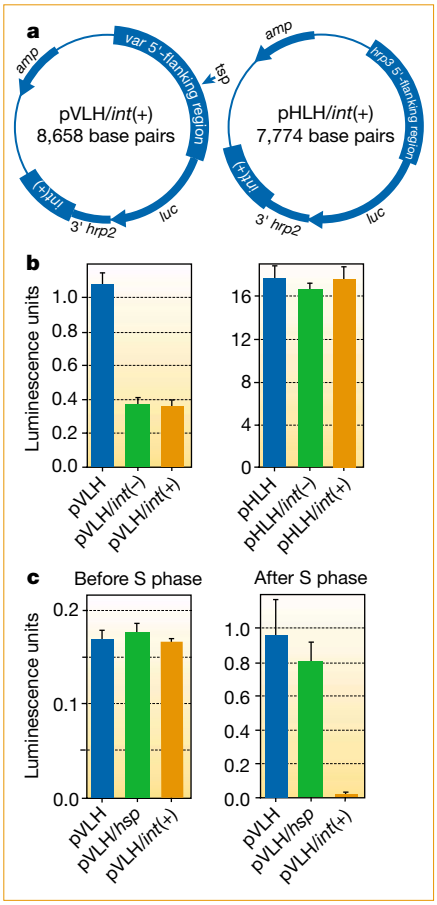

Figure 1 The role of cooperative control elements in S-phase-dependent silencing of *var* expression. **a,** Maps of pVLH/*int*(+) and pHLH/*int*(+). Plasmids pVLH/*int*(+), pVLH/*int*(−), pHLH/*int*(+) and pHLH/*int*(−) were constructed from the pVLH (pVLH-1) and pHLH (pHLH-1) plasmids[8] by inserting a *var* intron (*int*) in the (+) or (−) orientation downstream of the 3′-flanking region of the *hrp2* gene. Plasmid pVLH/*hsp* was obtained by inserting the intron sequence from the unrelated *P. falciparum hsp86* gene into pVLH at the same position. GenBank accession numbers for pVLH/*int*(+), pVLH/*hsp* and pHLH/*int*(+) sequences are AF338824, AF379854 and AF338825, respectively. The transcription start point (tsp) for *var* is indicated. **b,** Luciferase activity from *P. falciparum* parasites after spontaneous transformation with original and modified pVLH and pHLH plasmids. Assays were carried out after cultivation of parasites for 72 h in the presence of plasmid-loaded erythrocytes[10]. **c,** Luciferase activity from homogeneous plasmid populations before and after S phase in synchronized transfected parasites. Experiments were carried out in triplicate and repeated on at least three separate occasions. Error bars indicate calculated standard deviations.

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correction

An unusual social display in gorillas

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Nature **412,** 294 (2001)

Our claim that the manipulation of water for communicative means by western lowland gorillas had not previously been described in any wild primate was incorrect: we were unaware of observations of a small number of male chimpanzees in a community in Tanzania throwing stones into streams[1], apparently for the purpose of intimidation.

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